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Effect of Androgen Therapy and Anemia on Serum Erythropoietin Levels in Patients With Aplastic Anemia and Myelodysplastic Syndromes

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Immunoreactive serum erythropoietin (EPO) was measured in anemic and non-anemic patients with acquired non-severe aplastic anemia (AA; n = 22) and myelodysplastic syndromes (MDS; n = 31) receiving or not androgens to examine the effect of androgen therapy and anemia on EPO levels in these disorders. Soluble transferrin receptor (TfR) and absolute reticulocyte count (ARC) were also assayed in order to evaluate erythropoietic activity. AA and MDS patients were stratified for anemia and androgen treatment as follows: 12 untreated anemic patients; 17 anemic patients during androgen therapy; 14 non-anemic patients without any treatment (>1 year); and 10 non-anemic patients on androgen therapy. Although EPO levels in non-anemic patients were significantly higher than in healthy controls (n = 29) no statistically significant differences in Hb and EPO values were found between non-anemic patients receiving or not androgen therapy. In the linear regression analysis between Hb and log EPO concentration, no statistically significant differences in the slopes between untreated and androgen-treated anemic groups nor between both groups and patients with iron deficiency anemia (n = 23) were observed. However, the *y* intercept (log EPO) of regression line was significantly higher in androgen-treated anemic patients than in the androgen therapy-free anemic group. Serum TfR levels were higher in treated than in untreated anemic patients, whereas ARC was not different between both groups. These data seemingly indicate that (1) androgens at pharmacological doses do not increase serum EPO levels in non-anemic AA and MDS patients, and (2) in patients with AA and MDS, androgen-driven EPO stimulation is appreciably enhanced by anemia. Am. J. Hematol. 57:113–118, 1998. © 1998 Wiley-Liss, Inc.

Key words: erythropoietin; androgens; anemia; aplastic; myelodysplastic syndromes

INTRODUCTION

Erythropoietin (EPO) is the major cytokine factor for erythropoiesis. However, other growth factors and developmental agents, such as androgenic steroids, are also involved in erythrocyte production [1]. Several lines of investigation in animals and repeated clinical observations have shown that androgens stimulate erythropoiesis [2]. Pharmacologic doses of androgens are associated with an increase in red cell mass in both men and women [3] and have been employed in patients with aplastic anemia (AA) and myelodysplastic syndromes (MDS) with encouraging results [4–6].

It has been demonstrated that the administration of androgens to starved rodents and anemic patients results in a significant increase in the level of endogenous EPO [7–9]. Androgen-driven EPO stimulation is appreciably enhanced by hypoxia [10] and this effect may be due to an increase in sensitivity of the kidney to androgens [2]. Interestingly, in normal individuals pharmacologic or physiologic androgen-mediated red cell mass expansion has not been associated with any change in serum EPO [11,12].

In AA, it has been found that the abnormally high levels of EPO at diagnosis [13–16] decline along with

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TABLE I. Demographic Characteristics of Patients With Bone Marrow Failure Syndromes*

	All patients	No. of patients with				Age median (range)	Sex (M/F)
		AA	RA	RARS	RAEB		
Without androgens							
Anemic	12	3	6	0	3	34 (19–82)	4/8
Non-anemic	14	11	3	0	0	53 (20–90)	5/9
With androgens							
Anemic	17	3	9	3	2	35 (14–76)	10/7
Non-anemic	10	5	5	0	0	43 (20–74)	5/5

*AA, aplastic anemia; RA, refractory anemia; RARS, RA with ring sideroblasts; RAEB, RA with excess of blasts.

hemopoietic recovery. However, EPO levels in remission AA patients remain increased with respect to their hematocrit [17]. A wide range of serum EPO levels has been reported in patients with MDS [18] and it is suggested that the intensity of erythroid activity in the marrow, as well as the degree of anemia, may influence serum EPO concentration in these disorders [13,19,20].

Immunoreactive serum EPO levels were measured in anemic and non-anemic patients with acquired non-severe AA and MDS receiving or not androgens in order to understand better the effect of androgen therapy and anemia on EPO levels in these disorders. In addition, quantitation of soluble transferrin receptor (TfR) and absolute reticulocyte count (ARC) were performed to evaluate erythropoietic activity in AA and MDS.

PATIENTS AND METHODS

We studied 53 adult patients residing in Mexico City at 2,240 m above sea level (7,350 feet) with bone marrow failure syndromes: 22 with acquired non-severe AA and 31 with MDS: refractory anemia (RA), 23 patients; RA with ring sideroblasts (RARS), 3 patients; RA with excess of blasts (RAEB), 5 patients. Diagnosis and classification of AA and MDS were made according to well-established criteria [21,22]. Standard sucrose and acidified serum lysis tests were negative in all patients. All AA and MDS patients were categorized on the basis of whether or not they were receiving androgen therapy (oxymetholone or mesterolone, 2 mg/kg/day p.o.) and also on the basis of their Hb levels. The demographic characteristics of AA and MDS patients stratified for anemia and treatment are shown in Table I.

Study subjects included 12 anemic patients (Hb <14.5 g/dl in men and <13.0 g/dl in women) [23] at the time of diagnosis who had not received any treatment; 17 anemic patients who had received androgens for more than 6 months and were on this medication at the time of sampling; 14 non-anemic patients who achieved normal Hb levels only with androgens and had more than 1 year without any therapy; and 10 non-anemic patients who were currently receiving solely androgens due to the presence of leukopenia and/or thrombocytopenia. Androgen-treated patients received more packed red cell transfusions than untreated patients. At the time of the study, 10 patients were transfusion-dependent because of having Hb <8 g/dl. Twenty-three patients with iron deficiency anemia (IDA) and 29 apparently healthy subjects were also included in the study. Iron deficiency was established when patients had transferrin saturation index <15% and serum ferritin <20 μg/L in females and <37 μg/L in males [24].

Blood samples in all subjects were drawn during the fast period between 8:00 to 10:00 A.M. Serum was separated from the whole blood within 24 h and stored at −20°C until assayed. Blood count in EDTA-collected samples was analyzed in a Coulter (Hialeah, FL) Counter STKS. New methylene blue stain was employed to identify reticulocytes and was expressed in absolute numbers. Serum EPO and soluble TfR were assayed by duplicate using commercially available EIA kits. Serum of AA and MDS patients was diluted 1:60 or 1:80 to measure EPO with an EIA assay (BioMérieux, Lyon, France), which is based on a sandwich technique using two mouse monoclonal anti-EPO antibodies. Soluble TfR was measured in serum samples diluted 1:100 employing a microtiter plate (R&D Systems, Minneapolis, MN) coated with rabbit polyclonal antibody against TfR.

A linear regression analysis was performed to establish the relationship between EPO and Hb levels after $\log_{10}$ transformation of serum EPO concentration. Covariance analysis was used to compare slopes and *y*-intercepts of regression lines. Differences between groups were calculated with the U test of Mann-Whitney (one-tailed).

RESULTS

Hemoglobin values (median, range) in anemic patients without and with androgen therapy (9.9 g/dl, 6.0–14.1 g/dL; 8.0 g/dL, 3.9–12.4 g/dl, respectively) were similar to those found in IDA patients (10.9 g/dL, 6.6–12 g/dl). Serum EPO values (median, range) in anemic androgen-treated patients (3,728 mU/ml, 143–24,960 mU/ml) were significantly higher than in anemic androgen therapy-free patients (200 mU/ml, 36–12,962 mU/ml).

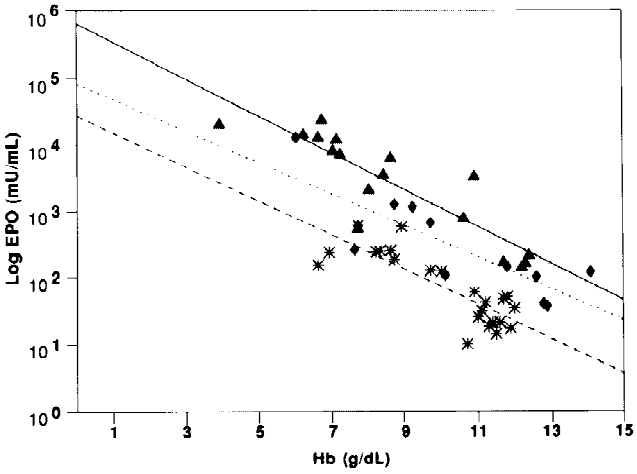

Fig. 1. The relation between Hb and log serum erythropoietin levels and equations for regression lines in 29 anemic AA and MDS patients: 12 androgen therapy-free patients (diamonds, dotted line), log EPO = −0.239*x* + 4.94 (r = −0.83, *P* < 0.0005); 17 androgen-treated patients (triangles, solid line), log EPO = −0.277*x* + 5.81 (r = −0.88, *P* < 0.0005); and 23 iron deficient patients (asterisks, dashed line), log EPO = −0.258*x* + 4.46 (r = −0.83, *P* < 0.0005).

Also, serum EPO levels were significantly higher in untreated and androgen-treated anemic patients than in the IDA group (50 mU/ml, 10–679 mU/ml). In order to establish the appropriateness of EPO response to the degree of anemia, a linear regression analysis and correlation coefficients between Hb concentration and serum log EPO level in anemic patients without and with androgen therapy and in IDA group were calculated (Fig. 1). Significant correlations were obtained between both parameters in anemic patients without and with androgen therapy ($r = -0.83$, $P < 0.001$ and $r = -0.88$, $P < 0.001$, respectively) and also in the IDA group ($r = -0.83$, $P < 0.001$). There were no statistically significant differences in the slopes of the regression lines between untreated and androgen-treated anemic patients nor between both groups of anemic patients and the IDA group, whereas the *y*-intercept of the regression line was significantly higher in androgen-treated anemic patients than in androgen therapy-free anemic patients ($P = 0.004$) and the IDA group ($P = 0.00001$). Also, the *y*-intercept in anemic patients not treated with androgens was significantly higher when compared with the IDA group ($P = 0.001$).

Hemoglobin values (median, range) in non-anemic patients without androgens (14.8 g/dl, 13.3–17 g/dl) and with androgens (14.6 g/dl, 13.3–17 g/dl) were similar to those recorded in healthy controls (16.4 g/dl, 13.8–18.6 g/dl). Serum EPO levels (median, range) in non-anemic patients without androgens (43 mU/ml, 15–238 mU/ml) and with androgens (86 mU/ml, 31–244 mU/ml) were significantly higher than in the control group (8 mU/ml; 4–28 mU/ml). No statistically significant differences in Hb and serum EPO levels between non-anemic patients without and with androgen therapy were found. Since it is widely assumed that in normal non-anemic healthy individuals the correlation between Hb and serum EPO is lost, we compared the relationship between these two parameters in non-anemic patients without and with androgen treatment with that obtained in non-anemic control subjects. Figure 2 depicts that whereas the relationship between Hb and log EPO in the control group was almost absent ($r = 0.04$) and in non-anemic androgen-treated patients was not significant ($r = -0.30$), this correlation coefficient was statistically significant in androgen therapy-free non-anemic patients ($r = -0.64$, $P < 0.01$).

As shown in Table II, serum TfR levels were significantly lower only in anemic patients not treated with androgens, whereas in the other three groups serum TfR values were not significantly different when compared with healthy controls. A significant increment in ARC was recorded in non-anemic patients regardless of androgen treatment, and ARC in both groups of anemic patients was not significantly different to that observed in the control group.

DISCUSSION

Employing an immunoassay we measured serum EPO in untreated and androgen-treated patients, residing at 2,240 m above sea level, with acquired non-severe AA and MDS who were or were not anemic at the time of the

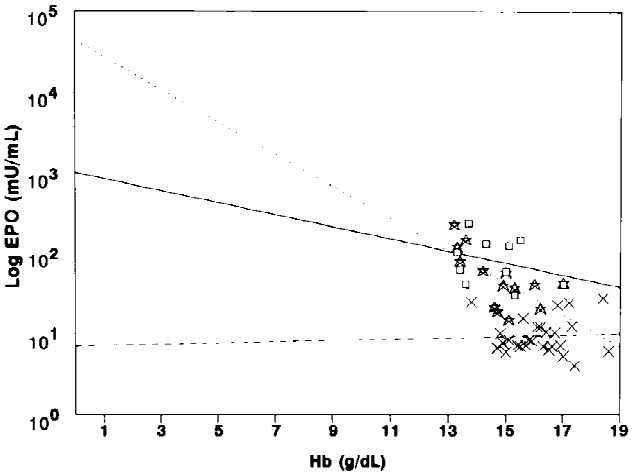

Fig. 2. The relation between Hb and log serum erythropoietin levels and equations for regression lines in 24 non-anemic AA and MDS patients: 14 androgen therapy-free patients (stars, dotted line), log EPO = −0.199*x* + 4.64 (r = −0.64, *P* < 0.01); 10 androgen-treated patients (squares, solid line), log EPO = −0.076*x* + 3.02 (r = −0.30, *P* = 0.4); and 27 healthy controls (crosses, dashed line), log EPO = 0.008*x* + 0.85 (r = 0.04, *P* = 0.8).

TABLE II. Median (Range) Values of Erythropoietin (EPO), Soluble Transferrin Receptor (TfR), and Absolute Reticulocyte Count (ARC) in Patients With Bone Marrow Failure Syndromes†

	EPO (mU/ml)	TfR (μg/ml)	ARC (10^9/L)
Without androgens			
Anemic			
AA [3]	588 (259–1,259)	1.32 (0.83–1.55)	10 (6–106)
RA [6]	103 (36–1,159)	2.07 (1.32–2.22)	92 (59–120)
RAEB [3]	663 (142–12,962)	1.01 (0.80–1.03)	6 (4–291)
All [12]	200 (36–12,962)*	1.44 (0.80–2.22)*	81 (4–291)
Non-anemic			
AA [11]	44 (19–238)	2.03 (1.22–2.72)	132 (35–215)
RA [3]	42 (15–152)	2.37 (2.14–2.62)	80 (30–104)
All [14]	43 (15–238)*	2.09 (1.22–2.72)	105 (30–215)*
With androgens			
Anemic			
AA [3]	12,600 (166–24,960)	1.49 (0.84–2.38)	29 (17–135)
RA [9]	3,474 (143–14,767)	1.87 (0.74–3.94)	51 (37–165)
RARS [3]	3,728 (562–21,603)	2.03 (1.70–4.63)	18 (7–78)
RAEB [2]	— (2,178–6,586)	— (1.05–5.85)	— (40–52)
All [17]	3,728 (143–24,960)*	1.87 (0.74–5.85)	42 (7–165)
Non-anemic			
AA [5]	129 (31–150)	2.43 (1.86–2.60)	111 (64–285)
RA [5]	64 (42–244)	2.29 (1.91–3.01)	198 (58–425)
All [10]	86 (31–244)*	2.30 (1.86–3.01)	123 (58–425)*
CONTROLS [29]	8 (4–28)	1.97 (1.50–2.64)	59 (21–131)

†AA, aplastic anemia; RA, refractory anemia; RARS, RA with ring sideroblasts; RAEB, RA with excess of blasts (number of subjects).
*$P < 0.05$ compared with controls.

study in order to investigate the effect of androgen therapy, anemia, and both on EPO levels in these diseases.

It has been shown that serum EPO values in mountaineers after 22 days of adaptation at 4,500 m were not different from values at sea level [25]. Immunoreactive serum EPO reference values at sea level reported by Cazzola and Beguin [26] (10–30 mU/ml) and Musto et al. [27] (5–30 mU/ml) are seemingly not different to those found in our normal healthy population (4–28 mU/ml). In consequence, apparently high altitude did not influence serum EPO concentrations in our patients with AA and MDS.

Our results showing that serum EPO values in non-anemic patients without and with androgen treatment were not statistically different are interpreted as indicating that, within the normal range of Hb, androgens seemingly do not increase serum EPO levels. This finding is consistent with a previous study [28] in which Hb values in pharmacologic castrated men decreased significantly, and after stopping the androgen antagonist hormone Hb and testosterone returned to pretreatment levels, whereas serum immunoreactive EPO levels did not change significantly during or after the period of androgen deprivation.

In the present study, data showing that serum log EPO levels adjusted for Hb were significantly higher in anemic androgen-treated patients than in anemic androgen therapy-free patients (Fig. 1) support the notion that androgen-driven EPO stimulation is appreciably enhanced by hypoxia [10]. These findings are in accordance with those published by Wolfson et al. [29] who demonstrated that concomitant use of androgens in dialysis patients resulted in a lower dose requirement for recombinant human EPO (rhEPO) when it was given intravenously. This information is in line with that reported by Ballal et al. [30] who found significantly higher hematocrit values in hemodialysis patients treated with 2,000 IU of rhEPO plus androgens three times a week than in those receiving only rhEPO at the same dose.

It is widely assumed that in normal healthy persons the correlation between Hb and serum log EPO is lost [17,31]. Herein, this correlation was absent in both healthy controls and in non-anemic androgen-treated patients, whereas non-anemic androgen therapy-free patients besides showing higher EPO levels than the control group also had an unexpected significant negative correlation between log EPO levels and Hb concentration. This finding has been previously observed in AA patients responding to immunosuppressive therapy [17]. However, it has not been reported to date that EPO levels in non-anemic MDS patients remain elevated for their Hb level when compared with healthy controls. The presence of normal Hb concentration with higher than expected EPO levels constitutes an argument against a simple feedback regulatory mechanism of EPO mediated through hypoxia. In line with this observation is the fact that in patients with relative or absolute erythroid hypoplasia, EPO levels higher than in patients with erythroid

hyperplasia and with the same degree of anemia have frequently been found [13,14,19,20]. Furthermore, specific therapy in patients with iron deficiency or pernicious anemia produces a decrease of EPO levels prior to any increase of hematocrit [19,32]. These observations suggest that a direct feedback mechanism of control between marrow cellularity and EPO synthesis could exist [33,34].

Soluble TfR is derived primarily from erythroid precursors in the bone marrow and its concentration in serum provides a reliable measurement of total erythroid activity [35,36] and ARC reflects effective erythropoiesis [37]. In the present study, untreated and androgen-treated non-anemic patients had identical Hb range levels and soluble TfR and ARC values were normal or increased in both groups, hence suggesting that both groups of non-anemic patients reached effective erythropoiesis.

As previously reported in severe AA [17], we found that anemic patients not treated with androgens had significantly lower TfR values than healthy controls. Intrinsic marrow hypoproliferation defined as low TfR levels, adequate EPO production, low hematocrit, and reduced production of reticulocytes [38,39] was more frequent in our anemic patients not treated with androgens (5 of 12; AA = 3, RAEB = 2) than in androgen-treated anemic patients (3/17; AA = 1, RARS = 2). Ineffective erythropoiesis characterized by increased TfR values and low ARC [40] was observed in 4 of our 17 anemic androgen-treated patients (RA = 2, RAEB = 1, RARS = 1), whereas any of the 12 anemic androgen therapy-free patients showed ineffective erythropoiesis. These data might explain why androgen-treated anemic patients had a median TfR value significantly higher than untreated anemic patients ($P < 0.05$) (Table II). In line with these findings are those reported by Musto et al. [27] and Cazzola et al. [41] who found ineffective erythropoiesis in 20–25% of MDS patients during rhEPO treatment.

In conclusion, overall data are interpreted as indicating that (1) androgens at pharmacologic doses apparently do not increase serum EPO levels in non-anemic AA and MDS patients, (2) in patients with AA and MDS, androgen-driven EPO stimulation is appreciably enhanced by anemia, and (3) androgen therapy seemingly promotes ineffective erythropoiesis in a certain number of anemic patients with MDS. Since administration of rhEPO has proven to be of benefit in some patients with AA and MDS [42], the use of androgens along with rhEPO could enhance the erythropoietic effect of this hormone, therefore, reducing the cost of treatment in these patients.

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REFERENCES

1. Spivak JL: The mechanism of action of erythropoietin. Int J Cell Cloning 4:139–166, 1986.
2. Shahidi NT: Androgens and erythropoiesis. N Engl J Med 289:72–80, 1973.
3. Neff MS, Goldberg J, Slifkin RF, Eiser AR, Calamia V, Kaplan M, Baez A, Gupta S, Mattoo N: A comparison of androgens for anemia in patients on hemodialysis. N Engl J Med 304:871–875, 1981.
4. Sánchez-Medal L, Gómez-Leal A, Duarte-Zapata L: Anabolic therapy in aplastic anemia. Blood 28:979, 1966.
5. Duarte L, Sandoval RL, Esquivel F: Androstane therapy of aplastic anaemia. Acta Haematol 47:140–145, 1972.
6. Romero-García F, Rosales-Hernández JJ: Empleo de la oximetolona a dosis bajas en el tratamiento de la anemia refractaria: Informe preliminar. Sangre 10:365–375, 1965.
7. Gurney CW, Fried W: Further studies on the erythropoietic effect of androgens. J Lab Clin Med 65:775–782, 1965.
8. Fried W, Gurney CW: Use of mild plethora to demonstrate an erythropoietic effect from small amounts of androgens. Proc Soc Exp Biol Med 120:519–523, 1965.
9. Mirand EA, Gordon AS, Wenig J: Mechanism of testosterone action in erythropoiesis. Nature 206:270–272, 1965.
10. Gordon AS, Mirand EA, Wenig J: Androgen actions on erythropoiesis. Ann NY Acad Sci 149:318–335, 1968.
11. Miller ME, Chandra M, Garcia JF: Clinical applications of measurement of serum immunoreactive levels of erythropoietin. Ann NY Acad Sci 459:375–381, 1985.
12. Hellebostad M, Haga P, Cotes PM: Serum immunoreactive erythropoietin in healthy normal children. Br J Haematol 70:247–250, 1988.
13. Pavlovic-Kentera V, Milenkovic P, Ruvidic R, Jovanovic V, Biljanovic-Paunovic L: Erythropoietin in aplastic anaemia. Blut 39:345–350, 1979.
14. Takeichi N, Umemura T, Nishimura J, Motomura S, Kozura M, Ibayashi H: Regulation of erythropoietin and burst-promoting activity production in patients with aplastic anaemia and iron deficiency anaemia. Acta Haematol 80:145–152, 1988.
15. Wognum AW, Lansdorp PM, Eaves AC, Krystal G: An enzyme-linked immunosorbent assay for erythropoietin using monoclonal antibodies, tetrameric immune complexes, and substrate amplification. Blood 74: 622–628, 1989.
16. Mason Garcia M, Beckman BS, Brookins JW, Powell JS, Lanham W, Blaisdell S, Keay L, Li SC, Fisher JW: Development of a new radioimmunoassay for erythropoietin using recombinant erythropoietin. Kidney Int 38:969–975, 1990.
17. Schrezenmeier H, Noé G, Raghavachar A, Rich IN, Heimpel H, Kubanek B: Serum erythropoietin and serum transferrin receptor levels in aplastic anaemia. Br J Haematol 88:286–294, 1994.
18. Hellström-Lindberg E: Efficacy of erythropoietin in the myelodysplastic syndromes: A meta-analysis of 205 patients from 17 studies. Br J Haematol 89:67–71, 1995.
19. de Klerk G, Rosengarten PCJ, Vet JWM, Goudsmit R: Serum erythropoietin (ESF) titres in anemia. Blood 58:1164–1170, 1981.
20. Jacobs A, Janowska-Wieczorek A, Caro J, Bowen DT, Lewis T: Circulating erythropoietin in patients with myelodysplastic syndromes. Br J Haematol 73:36–39, 1989.
21. Frickhofen N, Kaltwasser JP, Schrezenmeier H, Raghavachar A, Vogt HG, Herrman F, Freund M, Meusers P, Salama A, Heimpel H: Treatment of aplastic anaemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. N Engl J Med 324:1297–1304, 1991.

22. Bennett JM, Catovsky D, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of the myelodysplasic syndromes. Br J Haematol 51:189–199, 1982.
23. Piedras J, Reyes-Devesa S, Cordova MS, Chavez L: Límites de referencia de serie roja, obtenidos en el equipo Coulter S-Plus STKR, en adultos sanos residentes a 2,240 metros sobre el nivel del mar. Rev Invest Clin 43:174–178, 1991.
24. Alvarez X, Piedras J, Córdova MS, López-Karpovitch X, Cano R: Ferritina sérica en mujeres y varones. Valores de referencia. Rev Invest Clin 33:13–16, 1981.
25. Milledge JS, Cotes PM: Serum erythropoietin in humans at high altitude and its relation to plasma renin. J Appl Physiol 59:360–364, 1985.
26. Cazzola M, Beguin Y: New tools for clinical evaluation of erythron function in man. Br J Haematol 80:278–284, 1992.
27. Musto P, Modoni S, Alicino G, Savino A, Longo A, Bodenizza C, Falcone A, D'Arena G, Scalzulli P, Perla G, Casparrini G, Carotenuto M: Modifications of erythropoiesis in myelodysplastic syndromes treated with recombinant erythropoietin as evaluated by soluble transferrin receptor, high fluorescence reticulocytes and hypochromic erythrocytes. Haematologica 79:493–499, 1994.
28. Weber JP, Walsh PC, Peters CA, Spivak JL: Effect of reversible androgen deprivation on hemoglobin and serum immunoreactive erythropoietin in men. Am J Hematol 36:190–194, 1991.
29. Wolfson M, Mundt DJ, Hawley GG: Recombinant human erythropoietin utilization in Department of Veterans Affairs Dialysis Units. Am J Kidney Dis 24:184–191, 1994.
30. Ballal SH, Domoto DT, Polack DC, Marciulonis P, Martin KJ: Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. Am J Kidney Dis 17:29–33, 1991.
31. Ireland RM, Atkinson K, Concannon A, Dodds A, Downs K, Biggs JC: Serum erythropoietin changes in autologous and allogeneic bone marrow transplant patients. Br J Haematol 76:128–134, 1990.
32. Bjarnason I, Cotes PM, Knowles S, Reid C, Wilkins R, Peters TJ: Giant lymph node hyperplasia (Castleman's disease) of the mesentery. Observations on the associated anemia. Gastroenterology 87:216–223, 1984.
33. Stohlman F, Jr., Brecher G: Humoral regulation of erythropoiesis. V. Relationship of plasma erythropoietin level to bone marrow activity. Proc Soc Exp Biol Med 100:40–44, 1959.
34. McGonigle RJS, Ohene-Frempong K, Lewy JE, Fisher JW: Erythropoietin response to anaemia in children with sickle cell disease and Fanconi's hypoproliferative anaemia. Acta Haematol 74:6–9, 1985.
35. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA: Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 75:102–107, 1990.
36. Flowers CH, Skikne BS, Covell AM, Cook JD: The clinical measurement of serum transferrin receptor. J Lab Clin Med 114:368–377, 1989.
37. Hillman RS, Finch CA, eds. ''Red Cell Manual.'' Philadelphia: Davis, 1985.
38. Beguin Y, Clemons GK, Pootrakul P, Fillet G: Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood 81:1067–1076, 1993.
39. Bowen DT, Culligan D, Beguin Y, Kendall R, Willis N: Estimation of effective and total erythropoiesis in myelodysplasia using serum transferrin receptor and erythropoietin concentrations, with automated reticulocyte parameters. Leukemia 8:151–155, 1994.
40. Cazzola M, Ponchio L: Subcutaneous erythropoietin for treatment of refractory anemia in hematologic disorders. Blood 80:841–843, 1992.
41. Cazzola M, Ponchio L, Beguin Y, Rosti V, Bergamaschi G, Liberato NL, Fregoni V, Nalli G, Barosi G, Ascari E: Subcutaneous erythropoietin for treatment of refractory anemia in hematologic disorders. Results of a phase I/II clinical trial. Blood 79:29–37, 1992.
42. Bessho M, Jinnai I, Matsuda A, Saito M, Hirashima K: Improvement of anemia by recombinant erythropoietin in patients with myelodysplastic syndromes and aplastic anemia. Int J Cell Cloning 8:445–458, 1990.